

WE CLAIM:

*sub
a2*

1. A method of growing animal cells in fed batch cell culture comprising the steps of culturing the cells at a starting 5 osmolality of about 280-330 mOsm and controlling the glucose concentration in the cell culture to be between about 0.01 and 1g/L throughout the culturing.

10 2. A method of producing a polypeptide by animal cells comprising nucleic acid encoding the polypeptide in fed batch cell culture, comprising the steps of culturing the cells at a starting osmolality of about 400-600 mOsm and controlling the glucose concentration in the cell culture to be between about 0.01 and 1g/L throughout the culturing, wherein the starting cell density is at 15 least about 1.0×10^6 cells/mL.

10 3. A method of producing a polypeptide in cell culture comprising the steps of:

20 (a) in an initial growth stage, culturing animal cells comprising nucleic acid encoding the polypeptide at a starting osmolality of about 280-330 mOsm in the presence of a concentration of glucose controlled throughout the culturing to be within a range between about 0.01 and 1g/L, and

25 (b) in a production phase separate from step (a), inoculating the cultured animal cells of step (a) at a cell seed density of at least about 1.0×10^6 cells/mL; and

30 (c) culturing the animal cells at a starting osmolarity of about 400-600 mOsm in the presence of a concentration of glucose controlled throughout the culturing to be within a range between about 0.01 and 1g/L, inclusive.

4. The method of claim 3 wherein the glucose concentration is controlled in a range between about 0.02 and 0.5g/L for both steps (a) and (c).

5 5. The method of claim 4 wherein the glucose concentration is controlled in a range between about 0.05 and 0.2g/L for both steps (a) and (c).

10 6. The method of claim 3 wherein the osmolality is in the range 400-500 mOsm for step (c).

15 7. The method of claim 3 wherein the animal cells are cultured in the presence of a concentration of glutamine controlled throughout culturing steps (a) and (c) to be in a range between about 0.2 and 2mM.

8. The method of claim 3 wherein the culture medium contains excess amino acids.

20 9. The method of claim 8 wherein the amino acids are selected from the group consisting of Asn, Asp, Gly, Ile, Leu, Lys, Met, Ser, Thr, Trp, Tyr and Val.

25 10. The method of claim 3 wherein the cells are mammalian cells.

11. The method of claim 10 wherein the cells are Chinese Hamster Ovary (CHO) cells.

30 12. The method of claim 3 wherein the polypeptide is DNase.

13. The method of claim 12 wherein the osmolality in step (c) is about 400-500 mOsm.

14. The method of claim 3 wherein the polypeptide is TGF β .

15. The method of claim 14 wherein the osmolality in step (c) is about 400-450 mOsm.

5

16. The method of claim 3 wherein step (c) is terminated before the maximum polypeptide titer is obtained.

10 17. The method of claim 3 wherein step (c) is terminated

after 9 days or less.

18. DNase produced by the process of claim 17.

19. TGF β produced by the process of claim 17.

15 add a-1

DOCKET: 887-000000000000